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OBITUARY

Jeffrey G. Williams (1948-2022): a pioneer molecular biologist in development

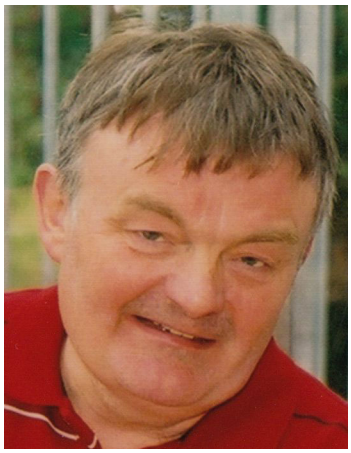
Robert R. Kay[1,*] and Cornelis J. Weijer[2,*]

Jeff Williams, who died earlier this year, was one of those lucky enough to enter the developmental biology field in the 1970s, just as it was emerging into the molecular age. Classical embryology had produced concepts – the organizer, morphogens and positional information being the most famous – that begged explanation at the molecular level. At the same time, nuclear transplantation experiments showed that differentiated cells retained the genetic information of their undifferentiated progenitors: cell differentiation was likely due to differential gene expression, not to loss of genetic material. Thus, classical questions took on a molecular edge: what were morphogens chemically (assuming they existed); did cells in embryos develop according to their position, or were other processes, such as cell-sorting, also important; and how were gene expression states established and stabilized over cell generations?

Jeff was an undergraduate at Kings College London and carried out his PhD at the Imperial Cancer Research Fund Laboratories (ICRF, now part of Cancer Research UK) in central London, where he studied drug resistance in normal and transformed cells with Ian MacPherson. He found that transformed cells were more resistant to actinomycin D (an inhibitor of RNA synthesis) than untransformed cells (Williams and MacPherson, 1973), seemingly because they could pump the drug out of the cell. Unwittingly, Jeff had touched on a fundamental issue in chemotherapy, which was not explicable until the discovery of multidrug resistance (MDR) efflux pumps in the plasma membrane.

For his post-doc, Jeff moved to the laboratory of Sheldon Penman at MIT, where he used bulk hybridization of cDNAs to investigate the mRNA complexity of cells in different states and to ask what made them different. Comparing transformed with non-transformed and growing with quiescent cells, he found that overall mRNA sequence complexity changed by less than 3% of the estimated 10,000 unique sequences present. With four papers in the newly established journal Cell, Jeff returned to the UK in a strong position to pursue his interest in gene expression.

Jeff chose to re-join the ICRF in 1975, but at their small laboratory in Mill Hill, on the outskirts of London. This laboratory, set in a tree-lined estate with a tennis court, was fully equipped for science to 1930s standards, but not to those of the 1970s. Fortunately, it was being revived and re-purposed by its Director, John Cairns, who had recently arrived from Cold Spring Harbor Laboratory, USA. For the next decade, before its closure, it was home to a remarkable flourishing of work in DNA repair and developmental biology, led by a cohort of young scientists appointed by John and his deputy, Julian Gross. For



Jeff Williams in 2005. Image credit: Tsuyoshi Araki (Sophia University, Japan).

developmental biology, it was ‘pick your organism time’ with (amongst others): Jonathan Slack, Les Dale and Jim Smith choosing amphibians; David Ish-Horovitz and Phil Ingham studying *Drosophila*; Brigid Hogan and Denise Barlow studying mice; and Julian Gross and Rob Kay focusing on *Dictyostelium*. Jeff brought a new element to this mix – he had followed his interest in gene expression by becoming an early adopter of gene-cloning technology.

When Jeff started his laboratory, the cloning of any gene was considered a potential hazard and so was carried out under tight safety precautions. There were no kits and few enzymes; reverse transcriptase came not from a catalogue, but as a gift from an American laboratory. Jeff proved a master of cloning, especially the crucial skills of cDNA and genomic library construction and screening. His early work included a detailed analysis of the globin genes from *Xenopus* with Roger Patient, collaborations on mouse genes with Brigid Hogan, and the sequencing of the human transferrin receptor. However, rather than diversity and dispersion, Jeff preferred to focus on one area and chose the slime mould *Dictyostelium discoideum*, perhaps because of the opportunity to study morphogenesis and its control through differential gene expression.

At this time, *Dictyostelium* was considered ideal for studying fundamental problems in development. Its development was relatively simple and easy to observe, it was amenable to biochemistry and genetics, and had a rapid life cycle. There were also two additional attractions: cyclic AMP and the ‘slug’. Cyclic AMP, identified by John Bonner and colleagues, is the chemoattractant that brings starving amoebae together into a multicellular mass at the start of development, and was considered as a candidate morphogen. The slug, which forms from this aggregated mass of cells, is patterned with the cells at the

[1]MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge, CB1 0QH, UK. [2]School of Life Sciences, University of Dundee, Dowstreet, Dundee, DD1 5EH, UK.

*Authors for correspondence (rrk@mrc-lmb.cam.ac.uk; c.j.weijer@dundee.ac.uk)

R.R.K., 0000-0003-2192-8150; C.J.W., 0000-0003-2192-8150

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front (prestalk cells) destined to make the stalk of the fruiting body, and those at the back (prespore cells), the spores. The tip of the slug is an organizer, which can induce a new axis when grafted to another slug, the body can regulate to form small slugs when cut into pieces, and there was evidence for both graded properties in the slug and cell sorting in its formation.

Jeff started his work on *Dictyostelium* with some of the first cloning of developmentally regulated genes in this organism. Initially, he cloned genes encoding abundant proteins, such as actin and the secreted lectin discoidin. Using probes for discoidin, Jeff, together with his PhD student Joan Devine, was able to show that, not only was the abundance of the mRNA developmentally regulated, but its transcription was too (Williams et al., 1979), confirming that the developmental programme was substantially driven by transcriptional changes.

With his work going well, Jeff moved to the new ICRF laboratory at Clare Hall, when the Mill Hill laboratories closed. Clare Hall developed great strength in DNA replication and repair, and colleagues included the Nobel laureates Tomas Lindahl and Tim Hunt. The next major initiative from Jeff's laboratory was to seek cell type-specific genes in order to help understand how cells make decisions between alternate pathways of differentiation. Meanwhile, Rob Kay, hunting for slime mould morphogens, had identified a chlorinated alkyl phenone called DIF as an inducer of stalk cells in culture experiments. By differential screening, Jeff and Keith Jermyn identified a pair of genes – *ecmA* and *ecmB* – expression of which was strongly induced by DIF and which they found were specific for prestalk cells (Williams et al., 1987). Later work showed they encoded extracellular matrix proteins.

Jeff (seated) with his long-term research associate Keith Jermyn at Clare Hall Laboratories. Image credit: Anon via Rob Kay (MRC Laboratory of Molecular Biology, UK).

Analysis of these genes and their regulation was to be a constant theme running throughout the rest of Jeff's career. There were immediate payoffs for our understanding of slug patterning. The *ecmA* and *ecmB* promoters, and elements derived from them, were linked to reporter proteins, and the expression of these was examined in slugs. To widespread surprise, the slug immediately got more complicated, as three subtypes of prestalk cells could be identified (Jermyn et al., 1989). It is now recognized that, as well as generating the classic precursors of the stalk of the mature fruiting body, the prestalk lineage provides cells that form cups supporting the spore mass, and a stabilizing disc at the base of the stalk.

One of the areas where *Dictyostelium* has led the way is in demonstrating that patterned tissues can be produced by cell sorting. Early experiments by others had shown that when slugs are stirred up, the mixed prestalk and prespore cells sort out and re-form perfectly patterned slugs, and that this can be guided by chemotaxis to cyclic AMP. However, a rigorous proof that sorting was the normal way of producing the prestalk/prespore pattern in the slug required definitive markers for prestalk and prespore cells, so that their early origin could be traced. Jeff and co-workers found that one type of prestalk cell – the pstA cells – first appear throughout the forming mound, intermingled with prespore cells. These cells then sort to the top to form a protruding tip of the prestalk zone (Williams et al., 1989). More recent work shows that the sorting mechanism involves both chemotaxis to cyclic AMP and adhesive interactions between cells mediated by allo-recognition. *Dictyostelium* is nothing if not versatile, and it appears that position is the governing factor in the differentiation of another type of prestalk cell, the pstB cells, which first appear in a block at the base of the mound (Early et al., 1995).

In 1994, Jeff was recruited as a founding member of the Laboratory of Molecular Cell Biology at University College London. He marked his induction to the Jodrell Chair with a public lecture, which he concluded on an idiosyncratic note by pulling out a guitar from under the podium and singing the audience a Beatles song! He was to repeat the same trick years later when awarded the Waddington medal by the British Society for Developmental Biology in 2004. Jeff was also honoured with membership of EMBO and the Royal Society of Edinburgh but is believed to have left his guitar at home for these awards. In his final career transition, Jeff moved to the University of Dundee in 1998, where he joined forces with Kees Weijer and Pauline Schaap to form a strong *Dictyostelium* axis.

Jeff next turned his attention to the signal transduction pathway used by DIF, which was completely unknown. With his colleagues, he worked systematically up from the *ecmA* and *ecmB* target genes, by first defining DIF-responsive promoter elements and then purifying the proteins that bound to them. In this way, Takefumi Kawata discovered the first STAT proteins outside metazoans (Kawata et al., 1997). STATs are activated by tyrosine phosphorylation; STATc becomes tyrosine phosphorylated in response to DIF and accumulates in the nucleus (Fukuzawa et al., 2001). However, *Dictyostelium* lacks true tyrosine kinases and the tyrosine phosphorylations are due to dual specificity kinases (Araki et al., 2012). Moreover, the regulated event is not phosphorylation, but dephosphorylation by the protein phosphatase PTP3, which is inhibited by DIF. STATc appears to be a repressor of DIF signalling; later work revealed activating routes leading to a bZIP transcription factor, DimB (Zhukovskaya et al., 2006), and a Myb transcription factor, MybE (Fukuzawa et al., 2006). A subsequent phospho-proteomics study also showed that dephosphorylation, and therefore activation of protein phosphatases, is a major response to DIF (Sugden et al., 2015). Sadly, Jeff was unable to reach the end of the DIF signalling pathway, and the DIF receptor remains for another generation to discover.

Jeff also played a major role in the *Dictyostelium* genome project, which arose in the 1990s as independent but cooperating genome projects based in Germany, the UK and the USA. The Sanger Centre hosted the UK project and provided key technologies to all. The genome, finally published in 2005 (Eichinger et al., 2005), opened a new chapter, facilitating almost all aspects of *Dictyostelium*

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research, supporting global approaches, such as microarrays and proteomics, and revealing many new gene families for investigation.

In his later years, Jeff continued his pursuit of transcriptional regulators controlling cell differentiation, helping to define two new classes. The CudA family, found in amoebae and plants, has an SH2 domain as well as a DNA-binding domain and is regulated by tyrosine phosphorylation, like STAT proteins (Yamada et al., 2008). The MRFs (myelin regulatory factors) are a new class of tethered transcription factors that are released by self-cleavage brought about by a viral protease domain included in their sequence (Senoo et al., 2013).

Jeff was a meticulous experimenter, and his youthful papers are object lessons in scientific self-doubt at work, with conclusions checked and cross-checked by different methods. Naturally, his laboratory produced the highest quality science. He was a fair but critical reviewer and as a colleague always helpful. His laboratory trained many good scientists who went on to enjoy diverse careers, including some who stayed with *Dictyostelium* for long periods.

Jeff enjoyed the *Dictyostelium* community and forged strong international links, both through collaborative EU grants and with a succession of Japanese researchers who came to his laboratory. He described the annual *Dictyostelium* conferences as like 'going on holiday with your friends' and several times he arranged Anglo-Japanese tennis matches, which were invariably won by the Japanese. One of Jeff's great pleasures was playing his guitar, often accompanied by his wife Natasha, who is a talented musician as well as a fellow scientist. Together they enjoyed Munro bagging – climbing 3000-foot-high mountains in Scotland – and Jeff also enjoyed playing sports socially, with soccer, squash, tennis, cricket and golf being among his favourites. To each he gave his utmost effort, while always being fun to play with. He was proudly Welsh and longed for the triumph of the Welsh rugby team, especially against the English.

Jeff is survived by Natasha and three children, two from a previous marriage. He is greatly missed as a fine scientist, a good friend and a pillar of the *Dictyostelium* community.

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